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Natural Resource Stewardship and Science

Aquatic Invertebrate Monitoring at Agate Fossil Beds National Monument

2019 Data Report

Natural Resource Data Series NPS/AGFO/NRDS—2022/XXXX

ON THIS PAGE
Deploying Hester-Dendy samplers at the Agate East site along the Niobrara River at Agate Fossil Beds National Monument.
Photograph courtesy by Tresize Tronstad, Wyoming Natural Diversity Database, University of Wyoming

ON THE COVER
Measuring stream discharge at the Agate Middle site along the Niobrara River at Agate Fossil Beds National Monument.
Photograph courtesy by Bryan Tronstad, Wyoming Natural Diversity Database, University of Wyoming

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Executive Summary

Monitoring ecosystems is vital to understanding trends over time and key to detecting change so that managers can address perturbations. Freshwater streams are the lifeblood of the surrounding landscape, and their health is a measure of the overall watershed integrity. Streams are the culmination of upland processes and inputs. Degradation on the landscape as well as changes to the stream itself can be detected using biota living in these ecosystems. Aquatic invertebrates are excellent indicators of ecosystem quality because they are relatively long-lived, sessile, diverse, abundant and their tolerance to perturbation differs. Aquatic invertebrates were monitored at three sites along the Niobrara River at Agate Fossil Beds National Monument in 2019 completing 23 years of data using Hester-Dendy and Hess samplers. Hess samplers are artificial multi-plate samplers suspended in the water column to allow invertebrates to colonize and Hess samples collect invertebrates in a known area on natural substrate and vegetation.

We identified 45 invertebrate taxa from four phyla (Annelida, Arthropoda, Mollusca, Nematoda) using both samplers in the Niobrara River (Appendix A and B). Hester-Dendy samplers collected 4 taxa not found in Hess samples and Hess samples collected 17 taxa not collected with Hester-Dendy samplers. Hess samples captured more (91%) than Hester-Dendy samples (62%). Crustacea, Diptera and Ephemeroptera were the most abundant groups of invertebrates collected in the Niobrara River. The proportion of Insecta, Annelida, Trichoptera and Diptera differed between Hester-Dendy and Hess samples ($p < 0.05$). EPT richness, proportion EPT taxa and Hilsenhoff's Biotic Index (HBI) ($p < 0.0001$) differed between sampler types, but taxa richness, taxa diversity and evenness ($p > 0.29$) did not. We collected the highest density of invertebrates at the Agate Middle site. Agate Spring Ranch had the lowest taxa richness and HBI, and the highest proportion of EPT taxa. HBI at the sites ranged from 4.0 to 6.3 (very good to fair from Hilsenhoff 1987) using the Hester-Dendy and 5.2 to 6.9 (good to fairly poor from Hilsenhoff 1987) using the Hess sampler.

Acknowledgments

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Introduction

Aquatic invertebrates are excellent indicators of ecosystem quality and they have been used to monitor aquatic ecosystems since the 1870s (Cairns and Pratt 1993). Managers and scientists use aquatic invertebrates to monitor ecosystem quality, because these animals have several characteristics that make them ideal for the task. Aquatic invertebrates are relatively long lived (weeks to >100 years) and remain in the water for most of their life (Rosenberg and Resh 1993b). Unlike water samples collected periodically, aquatic invertebrates live in the water year-round and represent conditions at that site. Water samples may miss discrete discharges of pollution, but aquatic invertebrates will respond to such events. These animals are also relatively sedentary making them ideal to assess water quality at a location. In addition, aquatic invertebrates are abundant, diverse, and easy to collect. Lower ecosystem quality can increase mortality, and decrease reproduction, survival and fitness of aquatic invertebrates (Johnson et al. 1993). The characteristic that makes aquatic invertebrates so successful at monitoring ecosystem quality is that some aquatic invertebrates are more sensitive to changes (i.e., stoneflies) while others are more tolerant (i.e., true flies; Barbour et al. 1999). Changes in the diversity or assemblage structure of aquatic invertebrates can be a sensitive measure of ecosystem quality (e.g., habitat degradation, changes in land use, altered food web, pollution), and these metrics are well-developed (Rosenberg and Resh 1993a).

The choice of what aquatic invertebrate sampler to use to monitor ecosystem quality can be a difficult decision that depends on many variables. All samplers have both advantages and disadvantages, but finding a sampler that minimizes bias and fulfills the objectives is critical. Bioassessment studies use a variety of sampling methods, including kicknets, fixed-area samplers (e.g., Hess sampler), artificial substrates (e.g., Hester-Dendy samplers) and dipnets (Carter and Resh 2001). Deciding what sampler to use often depends on characteristics of the ecosystem. For example, artificial substrates may be a good choice in aquatic habitats that are difficult to sample using other methods, such as large, deep rivers (De Pauw et al. 1986). The type of information needed plays a major role. For example, qualitative data may be sufficient if the study is estimating conditions, but more rigorous quantitative sampling is needed to assess change over time. Qualitative samplers report proportional data on the invertebrate assemblage, but fixed area samplers can provide quantitative information on the density and biomass of these animals. Artificial substrates can be a useful technique to collect aquatic invertebrates; however, the samples collected do not represent natural assemblages or densities and these samplers can be biased toward certain insect orders (Letovsky et al. 2012).

The National Park Service monitors three sites along the Niobrara River at Agate Fossil Beds National Monument since 1997. Our objective was to calculate bioassessment metrics from invertebrates collected using Hester-Dendy multiple plate samplers and a Hess sampler in 2019 to estimate changes over time. Our specific questions were 1.) Does the invertebrate assemblage and bioassessment metrics differ between samplers? 2.) Do bioassessment metrics differ among sites? and 3.) Which bioassessment metrics changed over time? We collected five Hester-Dendy and Hess samples at each of three sites to answer these questions. Data can be used to address management decisions in the park and understand how the river changed over time.

Study Area

The headwaters of the Niobrara River are located near Lusk, Wyoming and the river flows eastward into Nebraska and eventually into the Missouri River near Niobrara, Nebraska. The Niobrara River Basin covers 32,600 km^2 of which the majority is grassland in northern Nebraska (Galat et al. 2005). Over 95% of the land within the basin is used for agriculture. The Niobrara River flows through Agate Fossil Beds National Monument in western Nebraska about 23 km east of the Wyoming border. At this point the Niobrara River is a low order stream flowing through grassland. Agate Fossil Beds National Monument encompasses 2700 acres in a valley bottom, and 11 miles of river flows through the 4-mile-wide park (Figure 1). The riparian vegetation in the park is dominated by cattails (*Typha* sp.) and the invasive yellow flag iris (*Iris pseudacorus*). The substrate in the river predominantly consists of fine particles (e.g., sand, silt, and clay). Currently, northern pike (*Esox lucius*), white suckers (*Catostomus commersonii*) and green sunfish (*Lepomis cyanellus*) inhabit the river within the park; however, 9 other fish species were collected at Agate Fossil Beds National Monument prior to 1990 (Spurgeon et al. 2014).

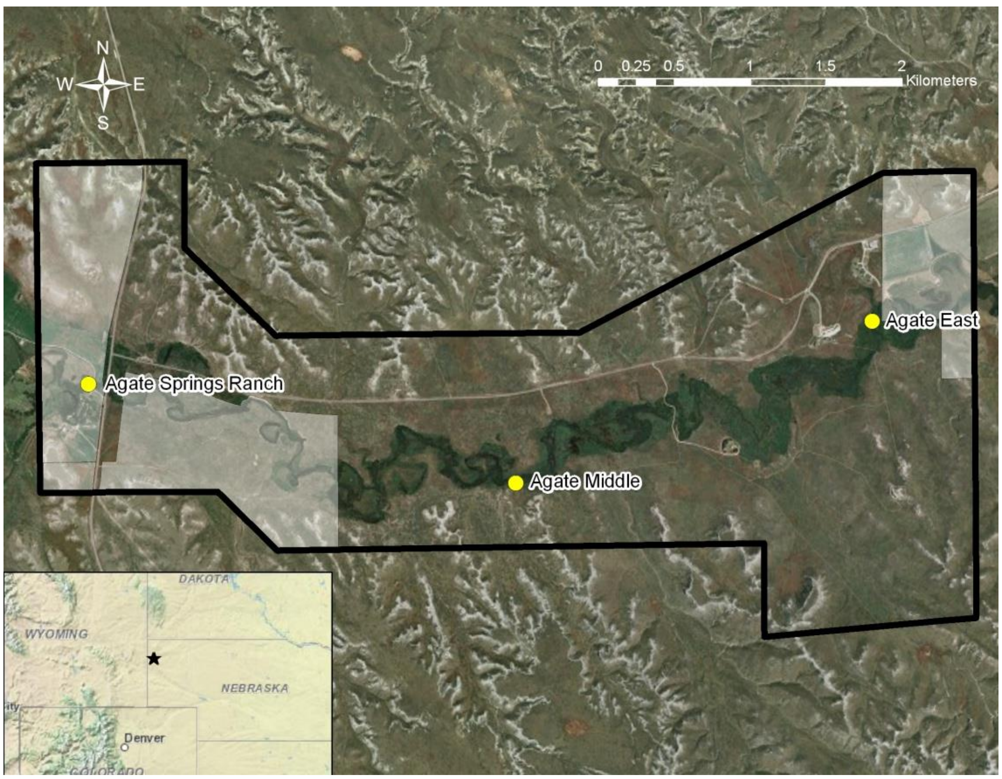

Figure 1. We sampled three sites along the Niobrara River at Agate Fossil Beds National Monuments. The black line is the Monument boundary and the transparent white areas are private land within the Monument boundary. The inset map shows the location of Agate Fossil Beds National Monument in Nebraska (star).

We sampled three sites along the Niobrara River at Agate Fossil Beds National Monument (Figures 1 and 2, Table 1). Aquatic invertebrates were first collected at Agate Springs Ranch in 1989 and again in 1996 when the Agate Middle site was added. A third site, Agate East, was added in 1997 and all sites have been monitored annually since that year. The most upstream site (Agate Springs Ranch) is located near the western park boundary. Agate Springs Ranch has an overstory of plains cottonwood (*Populus deltoides*) and cattails are more abundant than iris (Figure 2c). The central site, Agate Middle, lacks an overstory and has gravel substrate (Figure 2a). Both iris and cattails are abundant here. Finally, Agate East is located before the Niobrara River flows out of the park (Figure 2b). The riparian vegetation is dominated by iris with a few willows (*Salix* spp.) at Agate East. The river was treated in 2018 to reduce the iris growing there and riparian vegetation was recolonizing post-treatment in 2019.

Figure 2. Photos of (a) Agate Middle, (b) Agate East, (c) Agate Springs Ranch and (d) a colonized Hester-Dendy sampler in 2019.

Table 1. Location (Datum NAD83) of each site along the Niobrara River.

Coordinate	Ranch	Middle	East
Latitude	42.423	42.417	42.427
Longitude	-103.793	-103.759	-103.730
Elevation (m)	1354	1349	1343

Methods

General Measurements

We measured several variables to assess the general habitat characteristics of the Niobrara River including general water quality, water clarity, sediment composition, depth, and discharge. We measured dissolved oxygen concentration, pH, water temperature, specific conductivity and oxidation-reduction potential using a Yellow Springs Instruments Professional Plus. The instrument was calibrated on-site before use. We measured water clarity by estimating the depth at which a Secchi disk disappeared. The dominate substrate was assessed in the main channel and the bank of all sites using the soil texture test (Thien 1979). Clay was defined as fine particles forming a ribbon after removing water, whereas silt did not form a ribbon. Sand was characterized by particles 0.06-2 mm in diameter (sandpaper texture), gravel was 2-64 mm in diameter, cobble was 64-256 mm in diameter, boulder was 256-4000 mm in diameter, bedrock was >4000 mm in diameter, and hardpan/shale was firm, consolidated fine substrate. We recorded the location of each site using a global positioning system (GPS; Garmin Oregon 600). Finally, we estimated stream discharge (Q; ft^3/s) by measuring water depth (d; ft) and water velocity (v; ft/s) using a Marsh-McBirney Flo-Mate 2000 at 0.3 m intervals across the stream's width (w; ft) and summing each interval to calculate discharge using the following equation:

$$Q = \sum d_i \times v_i \times w_i$$

Hester-Dendy Samples

We deployed 7 Hester-Dendy samplers (76 mm by 76 mm, 9 plates, Wildlife Supply Company) at each site (Figure 2d). A rope was strung across the stream between two permanent posts and 7 loops were tied to separate the Hester-Dendy samplers. From each loop, another rope was tied with the Hester-Dendy samplers hanging at least 15 cm above the substrate. Debris dams were cleared from the samplers weekly. We retrieved the samplers after 30 days of colonization by approaching the site from downstream and placing a dipnet (150 µm mesh) under each sampler and cutting the rope. Hester-Dendy samplers were immediately placed in a container with water. Any organisms in the dip net were removed and placed in the same container. We dismantled and scrubbed the Hester-Dendy samplers to remove invertebrates that colonized the plates within 24 hours of collection, rinsed samples using a 212 µm sieve, and preserved samples in ~80% ethanol.

Hess Samples

We collected five Hess samples (500 µm mesh, 860 cm^2 sampling area, Wildlife Supply Company) from each site to sample invertebrates that lived in the emergent vegetation and sediment that is abundant along the margin of the Niobrara River. We placed the Hess sampler over cattails, iris, or bull rush (*Scirpus* sp.) to collect invertebrates that lived on the vegetation and in the surrounding sediment. The vegetation and sediment were vigorously agitated using our hands and a brush, and invertebrates were captured in the net of the Hess sampler. Samples were elutriated in the field, preserved in 80% ethanol and returned to the laboratory for analysis.

Invertebrate analysis

Invertebrates were sorted from debris in white trays and identified under a dissecting microscope. We rinsed each sample through 2 mm and either a 212 (Hester-Dendy samples) or 500 µm mesh sieves (Hess samples) to separate the larger and less abundant invertebrates from the smaller and more abundant invertebrates. All invertebrates were removed and identified in the larger (>2 mm) portion of the sample. If invertebrates were numerous in the smaller sieve, we subsampled the contents using the record player method (Waters 1969). Invertebrates were identified under a dissecting microscope using Merritt et al. (2008) for insects, and Thorp and Covich (2010) for non-insect invertebrates. Invertebrate tolerance values were assigned to taxa from Barbour et al. (1999). Density was calculated as the area sampled by the Hess sampler (0.086 m^2) or the surface area of the Hester-Dendy plates (0.10 m^2).

Six bioassessment metrics have been calculated since 1989 to estimate ecosystem quality based on the invertebrates collected: taxa richness, taxa diversity (Shannon's index), taxa evenness (taxa diversity divided by natural log of taxa richness), Ephemeroptera, Plecoptera and Trichoptera (EPT) richness, proportion of EPT taxa (number of EPT taxa divided by the total number of taxa collected) and Hilsenhoff's Biotic Index (HBI) for each replicate sample (Bowles et al. 2013). We used ANOVA to compare invertebrate assemblage structure (e.g., density, proportion of a taxon) and bioassessment metrics among sites and sampler types. If sites were significantly different ($\alpha < 0.05$), we used Tukey's honest significant difference (HSD) to verify which sites differed from one another. We used functional data analysis (FDA) to analyze long-term bioassessment metrics for trends. We plotted bioassessment metrics against time and calculated slopes and standard errors (SE) for each site. Slopes and SEs were averaged for each metric and site, and confidence intervals were calculated for each average slope. Trends were significant when the confidence interval did not include zero. We calculated invertebrate abundances, densities, proportions and bioassessment metrics using R (R Development Core Team 2013) and the packages *plyr* (Wickham 2011), *Matrix* (Bates and Maechler 2013), and *vegan* (Oksanen et al. 2013).

Results

Generally, conditions were similar among sites and visits. Dissolved oxygen peaked at 7.57 mg/L during the day and the minimum values reached 5.21 mg/L at night during August; however, minimum values were lower in July (Figure 3a, b). Water temperature varied between 19.9 and 24.7°C (Figure 3c, d). Specific conductivity was higher in July than August and pH was highest at the Agate Springs Ranch (Table 2). Reducing conditions dominated all sites (oxidation-reduction potential < 200 mV). The stream channel at Agate East was deepest and Agate Middle was narrowest. Discharge was highest at the Agate East and higher in July. Water clarity was lower in August (shallower Secchi disk depths). Channel substrate was dominated by fines and gravel, and bank substrate was dominated by sand and silt.

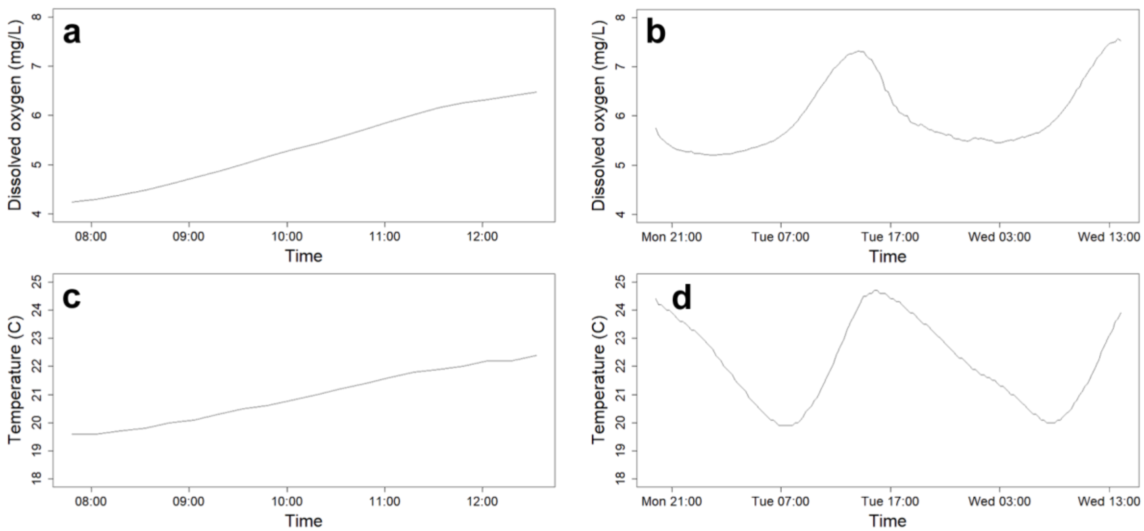

Figure 3. Dissolved oxygen (a, b) and water temperature (c, d) on 3 July 2019 when Hester-Dendy samplers were deployed (a, c) and 5-7 August 2019 when samplers were retrieved (b, d).

Table 2. Water quality and site characteristics measured when Hester-Dendy samplers were deployed (July), and retrieved (August). A "B" in front of Secchi disk depth indicated that we could see the stream bottom and the number is the maximum depth at the site. Channel width is the width of the channel without emergent vegetation.

Parameter	Units	Ranch	Middle	East	Ranch	Middle	East
Date	n/a	3-Jul-19	3-Jul-19	3-Jul-19	6-Aug-19	6-Aug-19	6-Aug-19
Start time	n/a	11:40	10:30	8:00	14:40	12:00	8:00
Water temperature	°C	21.4	20.7	19.5	24.4	22.5	20
Dissolved oxygen	% saturation	85	67	45	78	79.4	55
Dissolved oxygen	mg/L	6.4	5.1	3.6	6.47	6.84	5.0
Specific conductivity	µS/cm	720.0	626.0	733.0	397.2	400.1	408.5

Table 2 (continued). Water quality and site characteristics measured when Hester-Dendy samplers were deployed (July), and retrieved (August). A "B" in front of Secchi disk depth indicated that we could see the stream bottom and the number is the maximum depth at the site. Channel width is the width of the channel without emergent vegetation.

Parameter	Units	Ranch	Middle	East	Ranch	Middle	East
pH	n/a	8.04	8.08	7.94	8.12	7.92	7.86
ORP	mV	19.9	32.5	44.1	–	–	–
Secchi disk depth	cm	60	73(B)	87(B)	47	67.5	53
Max depth	ft	3.1	2.4	3.75	2.9	2.3	3.7
Width	ft	10.3	9.4	9.6	11.7	11.0	12.1
Discharge	ft^3/s	13.9	16.9	20.4	13.2	14.1	15.5
Dominant substrate	n/a	Sand	Gravel	Sand	Silt	Gravel	Sand
Air temperatures	°C	29	27	21	33	32	27

We identified 45 invertebrate taxa from four phyla (Annelida, Arthropoda, Mollusca, Nematoda) using both samplers in the Niobrara River (Appendix A and B). Hester-Dendy samplers collected 4 taxa not found in Hess samples and Hess samples collected 17 taxa not collected with Hester-Dendy samplers (Appendix A and B). The proportion of Insecta (F = 34, df = 1, p < 0.0001; Figure 4a), non-insect invertebrates (F = 34, df = 1, p < 0.0001), Annelida ($F = 9.0$, $df = 1$, $p = 0.006$; Figure 4b), Trichoptera ($F = 19.6$, $df = 1$, $p = 0.0001$; Figure 4i) and Diptera ($F = 11.4$, $df = 1$, $p = 0.002$; Figure 4e) differed among samplers. Proportions of Mollusca ($F = 0.6$, $df = 1$, $p = 0.45$; Figure 4c), Crustaceans ($F = 0.46$, $df = 1$, $p = 0.50$; Figure 4d), Coleoptera ($F = 2.0$, $df = 1$, $p = 0.17$; Figure 4e), Ephemeroptera ($F = 3.9$, $df = 1$, $p = 0.06$; Figure 4h), Odonata ($F = 2.0$, $df = 1$, $p = 0.17$; Figure 4g) and Hemiptera ($F = 1.9$, $df = 1$, $p = 0.18$) did not differ between samplers. Taxa richness ($F = 0.06$, $df = 1$, $p = 0.82$; Figure 5a), taxa diversity ($F = 0.81$, $df = 1$, $p = 0.81$; Figure 5b) and evenness ($F = 1.1$, $df = 1$, $p = 0.30$; Figure 5c) did not vary between sampler types, but EPT richness ($F = 23.9$, $df = 1$, $p < 0.0001$; Figure 6a), proportion EPT taxa ($F = 27.2$, $df = 1$, $p < 0.0001$; Figure 6b) and HBI ($F = 28.8$, $df = 1$, $p < 0.0001$; Figure 6c) differed between Hester-Dendy and Hess samples.

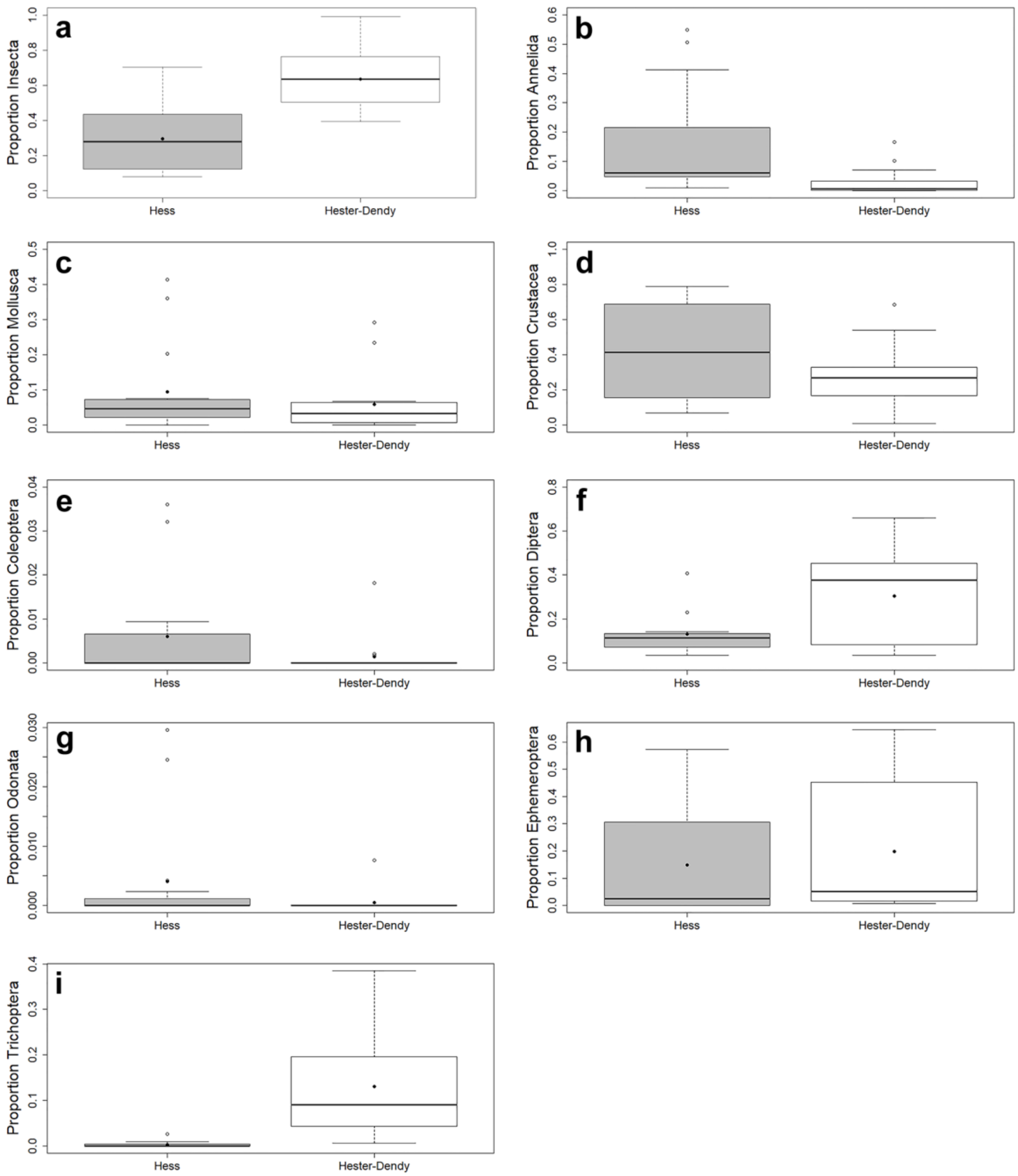

Figure 4. The proportion of (a) Insecta, (b) Annelida (c) Mollusca, (d) Crustacea, (e) Coleoptera, (f) Diptera, (g) Odonata, (h) Ephemeroptera, (i) and Trichoptera collected by the two sampler types. Black circles represent mean values and bold lines are median values, lower and upper limits of the box are the 25th and 75th percentiles, whiskers indicate the lower and upper limits of the data excluding outliers and open circles are outliers.

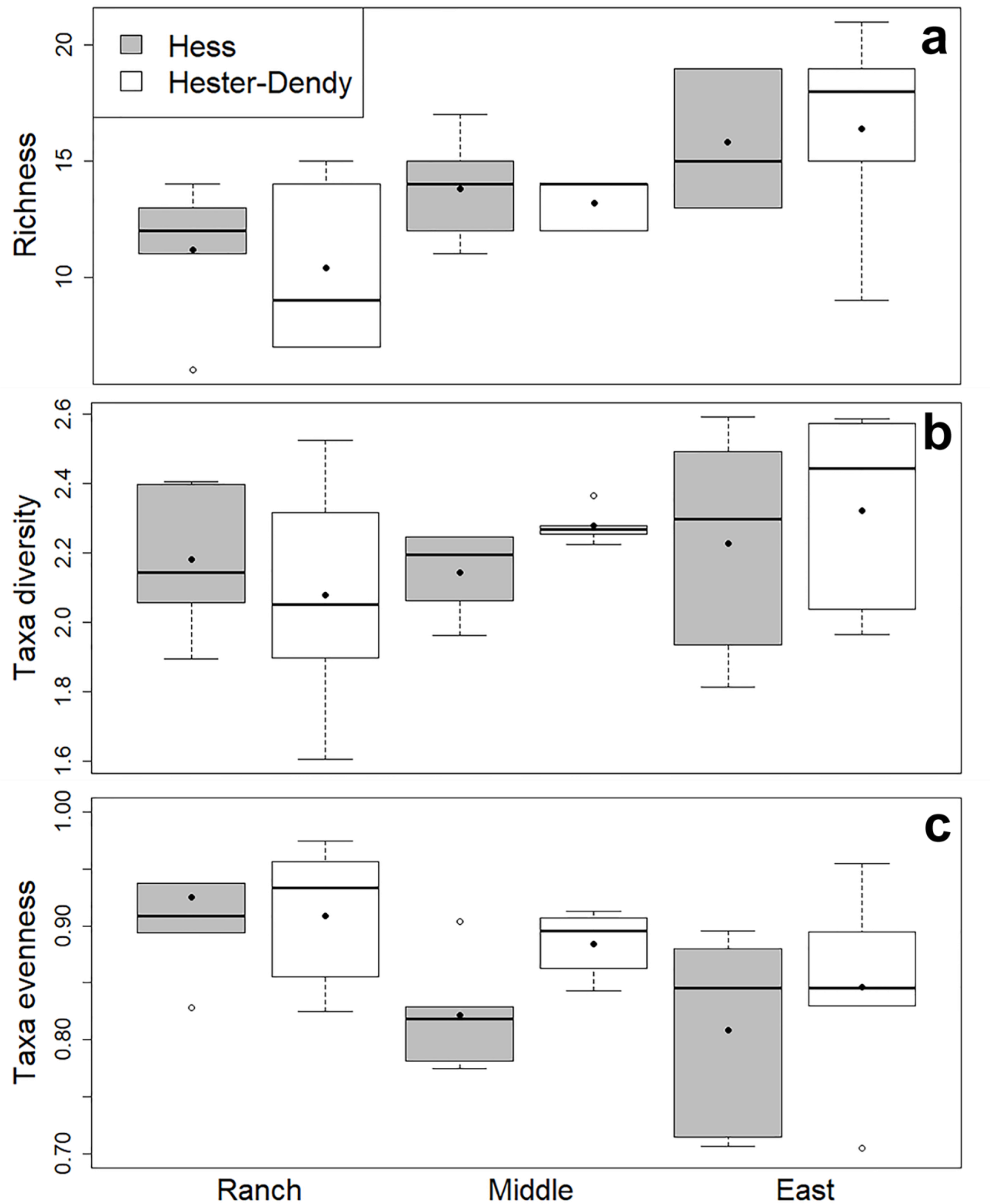

Figure 5. (a) Taxa richness, (b) taxa diversity and (c) taxa evenness calculated from Hess and Hester-Dendy samples collected from the Niobrara River in 2019. Higher values of all metrics indicate better ecosystem quality. Black circles represent mean values and bold lines are median values, lower and upper limits of the box are the 25th and 75th percentiles, whiskers indicate the lower and upper limits of the data excluding outliers and open circles are outliers.

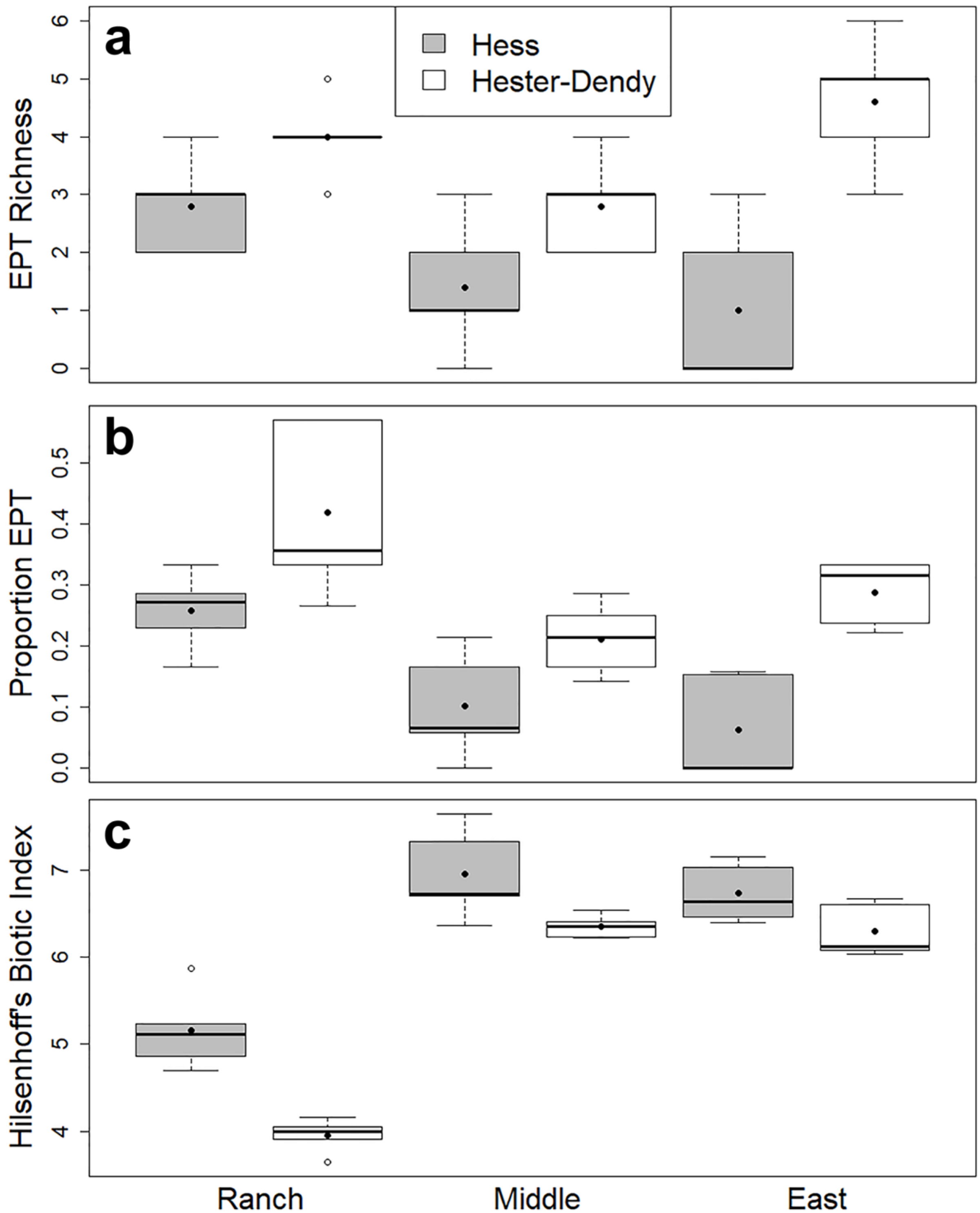

Figure 6. (a) Ephemeroptera, Plecoptera and Trichoptera (EPT) richness, (b) proportion of EPT taxa and, (c) Hilsenhoff's biotic index calculated from Hess and Hester-Dendy samples collected from the Niobrara River in 2019. Higher values of all metrics except HBI indicate better ecosystem quality. Black circles represent mean values and bold lines are median values, lower and upper limits of the box are the 25th and 75th percentiles, whiskers indicate the lower and upper limits of the data excluding outliers and open circles are outliers.

Using Hester-Dendy samplers, we collected 62% of the invertebrate taxa detected in the Niobrara River in 2019. Overall, more insects (62%) were collected with Hester-Dendy samplers than non-insect invertebrates. Insecta and Crustacea (88% of individuals) were the most numerous invertebrate groups followed by Gastropoda and Annelida in Hester-Dendy samples. Of the insects, Diptera, Trichoptera and Ephemeroptera were by far the most abundant in decreasing order of abundance (Appendix A). Hester-Dendy samples from Agate Middle (678 ind/sample; 6782 ind/m^2) contained more invertebrates than Agate Springs Ranch (201 ind/sample; 2006 ind/m^2) and Agate East (338 ind/sample; 3376 ind/m^2; $F = 11.6$, $df = 2$, $p = 0.002$; Tukey HSD, $p < 0.016$). Taxa richness was lowest at Agate Springs Ranch ($F = 3.6$, $df = 2$, $p = 0.06$; Table 3) and highest at Agate East (Tukey's HSD, $p = 0.05$). Neither taxa diversity ($F = 1.14$, $df = 2$, $p = 0.35$) nor taxa evenness ($F = 1.1$, $df = 2$, $p = 0.36$) differed among sites. Agate East had the most EPT taxa and Agate Middle had the fewest ($F = 5.0$, $df = 2$, $p = 0.03$; Tukey's HSD, p = 0.02). Agate Springs Ranch had the highest proportion of EPT taxa and Agate Middle samples had the lowest proportion ($F = 6.3$, $df = 2$, $p = 0.01$; Tukey's HSD, p = 0.01). The average tolerance value for an invertebrate in the assemblage was lowest at Agate Springs Ranch (HBI; $F = 189.4$, $df = 2$, $p < 0.001$; Tukey HSD, $p \leq 0.0001$).

Table 3. Mean invertebrate bioassessment metrics and standard errors at each site along the Niobrara River collected with Hester-Dendy and Hess samplers in 2019.

Sample Type	Metric	Ranch	Middle	East
Hester-Dendy samples	Taxa richness	10.4±1.72	13.2±0.49	16.4±2.09
	Taxa diversity	2.08±0.16	2.28±0.02	2.32±0.13
	Taxa evenness	0.91±0.03	0.88±0.01	0.85±0.04
	EPT richness	4.0±0.32	2.8±0.37	4.6±0.51
	Proportion EPT taxa	0.42±0.06	0.21±0.03	0.29±0.02
	HBI	4.0±0.09	6.3±0.06	6.3±0.14
Hess samples	Taxa richness	11.2±1.40	13.8±1.07	15.8±1.36
	Taxa diversity	2.18±0.10	2.14±0.06	2.22±0.15
	Taxa evenness	0.93±0.04	0.82±0.02	0.81±0.04
	EPT richness	2.8±0.37	1.4±0.51	1.0±0.63
	Proportion EPT taxa	0.26±0.03	0.10±0.04	0.06±0.04
	HBI	5.2±0.20	6.9±0.23	6.7±0.15

We collected 91% of invertebrate taxa detected using a Hess sampler in the Niobrara River. Overall, non-insect invertebrates (76%) dominated the invertebrate assemblage. Crustacea, Insects, Annelida and Gastropods (98% of individuals) were the most numerous invertebrate groups in decreasing order of abundance. Of the insects, Diptera (49%) were the most abundant followed by Ephemeroptera (43%), Coleoptera (3.4%), Odonata (2.3%) and Trichoptera (2.0%; Appendix B). Hess samples from Agate Middle (339 ind/sample; 3949 ind/m^2) had more invertebrates compared to Agate East (254 ind/sample; 2952 ind/m^2) and Agate Springs Ranch (212 ind/sample; 2471 ind/m^2), but densities did not differ among sites ($F = 0.70$, $df = 2$, $p = 0.52$). Taxa richness was lowest at Agate Springs Ranch and highest at Agate East ($F = 3.2$, $df = 2$, $p = 0.07$; Table 3). Taxa diversity did not vary among sites (F = 0.15, df = 2, p = 0.87). Taxa evenness was highest at Agate Springs Ranch (F = 3.4, df = 2, p = 0.07). Agate East had the fewest EPT taxa and Agate Springs Ranch had the most EPT taxa (F = 3.4, df = 2, p = 0.07). Agate Springs Ranch had the highest proportion of EPT taxa (F = 8.5, df = 2, p = 0.005; Tukey's HSD, p < 0.03). Additionally, invertebrates at Agate Springs Ranch had the lowest mean tolerance value (HBI; F = 24.4, df = 2, p < 0.0001; Tukey's HSD, $p < 0.001$).

Further Analysis

This 2019 data report is intended to provide a basic review of the data collected during the AGFO Invertebrate Monitoring. All invertebrate data found in this report is available from NGPN, as well as the archives found in the IRMA Data Store DataStore - Generic Dataset - (Code: 2283715) (nps.gov). For information on differences in sampling methods, invertebrate community trends over time, and potential impacts to the macroinvertebrate community at AGFO, the 2010-2019 Aquatic Invertebrate, Water Quality, and Riparian Vegetation Status and Trends for Agate Fossil Beds National Monument report will provide in-depth information on those topics.

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Appendix A

Table A-1. Mean density (ind/m2) of invertebrates collected from three sites along the Niobrara River at Agate Fossil Beds National Monument in 2019 using Hester-Dendy samplers.

Taxa Level 1	Taxa Level 2	Taxa Level 3	Taxa Level 4	East	Middle	Ranch
Annelida	–	–	–	240	44	295
	Hirudinea	–	–	135	0	0
	Oligochaeta	–	–	105	44	295
Arachnida	–	–	–	55	55	20
	Trombidiformes (Acari)	–	–	55	55	20
Crustacea	–	–	–	1387	1672	349
	Amphipoda	–	–	1028	1492	199
	Amphipoda	Gammaridae	–	342	212	83
	Amphipoda	Gammaridae	*Gammarus*	342	212	83
	Amphipoda	Hyalellidae	–	686	1280	116
	Amphipoda	Hyalellidae	*Hyalella*	686	1280	116
	Cladocera	–	–	10	10	0
	Copepoda	–	–	273	140	140
	Copepoda	Harpacticoida	–	273	140	140
	Decapoda	–	–	10	0	0
	Decapoda	Cambaridae	–	10	0	0
	Decapoda	Cambaridae	*Orconectes neglectus*	10	0	0
	Ostracoda	–	–	67	30	10

Table A-1 (continued). Mean density (ind/m2) of invertebrates collected from three sites along the Niobrara River at Agate Fossil Beds National Monument in 2019 using Hester-Dendy samplers.

Taxa Level 1	Taxa Level 2	Taxa Level 3	Taxa Level 4	East	Middle	Ranch
Mollusca	–	–	–	0	0	0
	Gastropoda	–	–	141	931	20
	Gastropoda	Ancylidae	–	104	896	10
	Gastropoda	Lymnaeidae	–	0	10	0
	Gastropoda	Physidae	–	37	25	10
Insecta	–	–	–	1892	4213	1786
	Coleoptera	–	–	10	0	20
	Coleoptera	Dytiscidae	–	0	0	10
	Coleoptera	Dytiscidae	*Hygrotus*	0	0	10
	Coleoptera	Dytiscidae	*Elmidae*	10	0	0
	Coleoptera	Dytiscidae	*Lara*	10	0	0
	Coleoptera	Hydrophilidae	–	0	0	10
	Diptera	–	–	1264	3606	190
	Diptera	Ceratopogonidae	–	42	0	50
	Diptera	Ceratopogonidae	*Bezzia/Palpomyia*	17	0	40
	Diptera	Ceratopogonidae	*Culicoides*	25	0	10
	Diptera	Chironomidae	–	795	2386	82
	Diptera	Chironomidae	Chironomidae (pupae)	47	146	0
	Diptera	Chironomidae	Non-Tanypodinae	748	2240	82
	Diptera	Chironomidae	Tanypodinae	294	256	28

Table A-1 (continued). Mean density (ind/m2) of invertebrates collected from three sites along the Niobrara River at Agate Fossil Beds National Monument in 2019 using Hester-Dendy samplers.

Taxa Level 1	Taxa Level 2	Taxa Level 3	Taxa Level 4	East	Middle	Ranch
Insecta (continued)	Diptera	Empididae	–	43	10	0
	Diptera	Empididae	Hemerodromia	23	10	0
	Diptera	Simuliidae	–	80	954	30
	Diptera	Simuliidae	*Simulium*	80	944	30
	Diptera	Stratiomyiidae	Stratiomyiidae (all)	10	0	0
	Diptera	Stratiomyiidae	*Odontomyia*	10	0	0
	Ephemeroptera	–	–	178	143	1090
	Ephemeroptera	Baetidae	–	13	58	68
	Ephemeroptera	Baetidae	*Baetis*	13	58	68
	Ephemeroptera	Caenidae	–	13	15	0
	Ephemeroptera	Caenidae	*Caenis*	13	15	0
	Ephemeroptera	Ephemeridae	–	0	0	60
	Ephemeroptera	Ephemeridae	*Hexagenia*	0	0	60
	Ephemeroptera	Heptageniidae	–	23	0	546
	Ephemeroptera	Heptageniidae	*Heptagenia*	23	0	546
	Ephemeroptera	Leptophlebiidae	–	128	70	416
	Ephemeroptera	Leptophlebiidae	*Paraleptophlebia*	128	70	416
	Odonata	–	–	40	0	0
	Trichoptera	–	–	400	464	486
	Trichoptera	Hydropsychidae	–	380	464	486

Table A-1 (continued). Mean density (ind/m2) of invertebrates collected from three sites along the Niobrara River at Agate Fossil Beds National Monument in 2019 using Hester-Dendy samplers.

Taxa Level 1	Taxa Level 2	Taxa Level 3	Taxa Level 4	East	Middle	Ranch
Insecta (continued)	Trichoptera	Hydropsychidae	*Cheumatopsyche*	380	464	486
	Trichoptera	Polycentropodidae	–	20	0	0
	Trichoptera	Polycentropodidae	*Polycentropus*	20	0	0
Nematoda	–	–	–	20	25	0

Appendix B

Table B-1. Mean density (ind/m2) of invertebrates collected from three sites along the Niobrara River at Agate Fossil Beds National Monument in 2019 using a Hess sampler.

Taxa Level 1	Taxa Level 2	Taxa Level 3	Taxa Level 4	East	Middle	Ranch
Annelida	–	–	–	478	186	840
	Hirudinea	–	–	18	105	12
	Oligochaeta	–	–	460	81	828
Arachnida	–	–	–	12	–	–
	Trombidiformes (Acari)	–	–	12	–	–
Mollusca	–	–	–	24	12	12
	Bivalvia	–	–	24	12	12
	Bivalvia	Sphaeriidae	–	24	12	12
	Gastropoda	–	–	187	856	70
	Gastropoda	Ancylidae	–	56	753	70
	Gastropoda	Lymnaeidae	–	79	82	–
	Gastropoda	Physidae	–	52	21	–
Collembola	–	–	–	68	21	12
Crustacea	–	–	–	1709	3376	412
	Amphipoda	–	–	1586	2044	285
	Amphipoda	Gammaridae	–	226	260	41
	Amphipoda	Gammaridae	*Gammarus*	226	260	41
	Amphipoda	Hyalellidae	–	1360	1784	244

Table B-1 (continued). Mean density (ind/m2) of invertebrates collected from three sites along the Niobrara River at Agate Fossil Beds National Monument in 2019 using a Hess sampler.

Taxa Level 1	Taxa Level 2	Taxa Level 3	Taxa Level 4	East	Middle	Ranch
Crustacea (continued)	Amphipoda	Hyalellidae	*Hyalella*	1360	1784	244
	Cladocera	–	–	20	–	–
	Copepoda	–	–	91	169	30
	Copepoda	Harpacticoida	–	91	169	30
	Decapoda	–	–	12	59	98
	Decapoda	Cambaridae	–	12	59	98
	Decapoda	Cambaridae	*Orconectes neglectus*	12	59	98
	Ostracoda	–	–	–	1105	–
Insecta	–	–	–	1219	841	1544
	Coleoptera	–	–	309	47	12
	Coleoptera	Dytiscidae	–	46	–	–
	Coleoptera	Dytiscidae	*Liodessus*	23	–	–
	Coleoptera	Gyrinidae	–	233	47	12
	Coleoptera	Gyrinidae	*Gyrinus*	233	47	12
	Coleoptera	Lampyridae	–	23	–	–
	Coleoptera	Melyridae	–	12	–	–
	Coleoptera	Staphlinidae	–	18	–	–
	Diptera	–	–	682	606	265
	Diptera	Ceratopogonidae	–	40	–	–
	Diptera	Ceratopogonidae	*Bezzia/Palpomyia*	12	–	–

Table B-1 (continued). Mean density (ind/m2) of invertebrates collected from three sites along the Niobrara River at Agate Fossil Beds National Monument in 2019 using a Hess sampler.

Taxa Level 1	Taxa Level 2	Taxa Level 3	Taxa Level 4	East	Middle	Ranch
Insecta (continued)	Diptera	Ceratopogonidae	*Culicoides*	12	–	–
	Diptera	Ceratopogonidae	*Probezzia*	16	–	–
	Diptera	Chironomidae	–	285	302	156
	Diptera	Chironomidae	Chironomidae (pupae)	19	27	12
	Diptera	Chironomidae	Non-Tanypodinae	265	275	144
	Diptera	Chironomidae	Tanypodinae	12	61	12
	Diptera	Empididae	–	–	12	–
	Diptera	Ephydridae	–	–	35	–
	Diptera	Ephydridae	*Notiphila*	–	35	–
	Diptera	Simuliidae	–	99	131	96
	Diptera	Simuliidae	*Simulium*	99	119	96
	Diptera	Stratiomyiidae	–	12	–	–
	Diptera	Stratiomyiidae	*Odontomyia*	12	–	–
	Diptera	Syrphidae	–	198	30	–
	Diptera	Tabanidae	–	12	24	–
	Diptera	Tabanidae	*Chrysops*		24	–
	Diptera	Tabanidae	*Tabanidae*	12		–
	Diptera	Tipulidae	–	24	12	–
	Diptera	Tipulidae	*Erioptera*	12	–	–
	Diptera	Tipulidae	*Helius*	12	12	–

Table B-1 (continued). Mean density (ind/m2) of invertebrates collected from three sites along the Niobrara River at Agate Fossil Beds National Monument in 2019 using a Hess sampler.

Taxa Level 1	Taxa Level 2	Taxa Level 3	Taxa Level 4	East	Middle	Ranch
Insecta (continued)	Ephemeroptera	–	–	53	82	1180
	Ephemeroptera	Baetidae	–	24	70	560
	Ephemeroptera	Baetidae	*Baetis*	24	70	560
	Ephemeroptera	Caenidae	–	12	–	23
	Ephemeroptera	Caenidae	*Caenis*	12	–	23
	Ephemeroptera	Ephemeridae	–	–	–	285
	Ephemeroptera	Ephemeridae	*Hexagenia*	–	–	285
	Ephemeroptera	Heptageniidae	–	18	12	311.25
	Ephemeroptera	Heptageniidae	*Heptagenia*	18	12	311.25
	Hemiptera	–	–	–	24	–
	Hemiptera	Corixidae	–	–	24	–
	Hemiptera	Corixidae	*Palmacorixa*	–	12	–
	Odonata	–	–	175	12	70
	Odonata	Aeshnidae	–	–	–	12
	Odonata	Aeshnidae	*Aeshna*	–	–	12
	Odonata	Coenagrionidae	–	12	–	–
	Odonata	Coenagrionidae	*Coenagrion/Enallagma*	12	–	–
	Trichoptera	–	–	–	70	18
	Trichoptera	Hydropsychidae	–	–	58	18
	Trichoptera	Hydropsychidae	*Cheumatopsyche*	–	58	18

Table B-1 (continued). Mean density (ind/m2) of invertebrates collected from three sites along the Niobrara River at Agate Fossil Beds National Monument in 2019 using a Hess sampler.

Taxa Level 1	Taxa Level 2	Taxa Level 3	Taxa Level 4	East	Middle	Ranch
Insecta (continued)	Trichoptera	Polycentropodidae	–	–	12	–
	Trichoptera	Polycentropodidae	*Polycentropus*	–	12	–
Nematoda	–	–	–	67	35	18

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